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Increased adrenal androgen levels in patients with Prader-Willi syndrome are associated with insulin, IGF-I, and leptin, but not with measures of obesity

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Abstract: **BACKGROUND/AIM:** Since hyperandrogenism in simple obesity is assumed to arise from hyperinsulinism and/or increased insulin-like growth factor I (IGF-I) or leptin levels, we examined how in patients with Prader-Willi syndrome (PWS), the most frequent form of syndromal obesity, the accelerated adrenarche can be explained despite hypothalamic-pituitary insufficiency with low levels of insulin and IGF-I. **METHODS:** In 23 children with PWS and a mean age of 5.6 years, height, weight, fat mass, fasting insulin concentration, insulin resistance (by HOMA-R; see text), and leptin and IGF-I levels were determined to test whether they explain the variance of the levels of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), of androstenedione, and of cortisol before and during 42 months of therapy with growth hormone. **RESULTS:** The baseline DHEAS, DHEA, and androstenedione concentrations were increased as compared with age-related reference values, whereas the cortisol level was always normal. During growth hormone treatment, the DHEA concentration further rose, and the cortisol level decreased significantly. The insulin and IGF-I concentrations were low before therapy, while fat mass and leptin level were elevated. The hormonal covariates provided alone or together between 24 and 60% of the explanation for the variance of adrenal androgen levels, but the anthropometric variables did not correlate with them. **CONCLUSIONS:** In children with PWS, elevated androgen levels correlate with hormones that are usually associated with adiposity. However, the lack of direct correlations between disturbed body composition and androgen levels as well as the increased sensitivity to insulin and IGF-I are abnormalities specific to PWS, potentially caused by the underlying hypothalamic defect.

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Increased Adrenal Androgen Levels in Patients with Prader-Willi Syndrome Are Associated with Insulin, IGF-I, and Leptin, but Not with Measures of Obesity

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Key Words

Prader-Willi syndrome · Adrenarche · Adrenal androgens · Obesity

Abstract

Background/Aim: Since hyperandrogenism in simple obesity is assumed to arise from hyperinsulinism and/or increased insulin-like growth factor I (IGF-I) or leptin levels, we examined how in patients with Prader-Willi syndrome (PWS), the most frequent form of syndromal obesity, the accelerated adrenarche can be explained despite hypothalamic-pituitary insufficiency with low levels of insulin and IGF-I. **Methods:** In 23 children with PWS and a mean age of 5.6 years, height, weight, fat mass, fasting insulin concentration, insulin resistance (by HOMA-R; see text), and leptin and IGF-I levels were determined to test whether they explain the variance of the levels of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), of androstenedione, and of cortisol before and during 42 months of therapy with growth hormone. **Results:** The baseline DHEAS, DHEA, and androstenedione concentrations were increased as compared with age-related reference values, whereas the cortisol level

was always normal. During growth hormone treatment, the DHEA concentration further rose, and the cortisol level decreased significantly. The insulin and IGF-I concentrations were low before therapy, while fat mass and leptin level were elevated. The hormonal covariates provided alone or together between 24 and 60% of the explanation for the variance of adrenal androgen levels, but the anthropometric variables did not correlate with them. **Conclusions:** In children with PWS, elevated androgen levels correlate with hormones that are usually associated with adiposity. However, the lack of direct correlations between disturbed body composition and androgen levels as well as the increased sensitivity to insulin and IGF-I are abnormalities specific to PWS, potentially caused by the underlying hypothalamic defect.

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Introduction

Childhood obesity is generally accompanied by accelerated growth [1] and early sexual development. A recent study conducted in 17,077 US girls confirmed that higher

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body mass index (BMI) z-scores were found in white girls with isolated pubic hair growth [2]. Early pubarche can result from a precocious increase of adrenal androgen levels present in obesity [3–5]. Adrenal stimulation, in turn, may be the consequence of hyperinsulinism and/or increased bioavailability of insulin-like growth factor I (IGF-I) [6–8]. In addition, leptin is reported to increase the adrenal activity in prepubertal children, e.g., via stimulation of corticotropin-releasing hormone [9], and leptin also stimulates the synthesis of adrenal androgens *in vitro* [10].

Prader-Willi syndrome (PWS) is the most frequent form of syndromal obesity. The phenotype is linked with a hypothalamic defect. To date, growth hormone (GH) insufficiency and hypogonadism are well described, and the gonadal development at puberty is typically delayed or incomplete. Surprisingly, in this context, premature adrenarche may be observed in children affected by PWS [11–15], although the insulin [16] and IGF-I [17] levels are below the normal average due to insufficient GH secretion [18]. The issue of accelerated adrenal development against a background of hypothalamic-pituitary insufficiency in PWS has not yet been explained. Although gross abnormalities of adrenal function have not been reported, the integrity of the hypothalamic-pituitary-adrenal axis needs to be confirmed by yet more sophisticated methods [19, 20]. Therefore, it has not yet been specified, whether premature adrenarche is the consequence of an underlying hypothalamic dysfunction or results from adiposity, as in healthy obese children [3, 4].

In order to test the hypothesis that premature adrenarche in PWS is caused by obesity and related metabolic alterations, independently of the hypothalamic regulation, we studied the relations between adrenal androgens and body composition parameters or associated hormones, such as insulin, IGF-I, and leptin, in children with PWS and the potential changes induced by treatment with GH. The cortisol levels were documented to assess alterations of the adrenal glucocorticoid axis.

Patients and Methods

In a prospective study, we examined a group of 23 children, 11 girls and 12 boys, with genetically proven PWS, in whom growth and body composition before and during long-term GH therapy (GHT) were previously described [21, 22]. At baseline, the mean age was 5.6 (range 0.5–14.6) years and the bone age 6.15 (range 0.6–14.6) years. Four girls and 1 boy had entered puberty at onset of therapy (breast stage 2–3 and genital stage 3, respectively). The observation period in 19 children (8 female, 11 male) was long enough to notice precocious pubic hair development during the critical age, but 4 adolescents

were presented after the age of 9 years, thus too late to observe precocious pubarche. Premature adrenarche was defined as precocious pubic hair growth before the age of 8 years in girls and of 9 years in boys and elevated adrenal androgen levels. The following parameters were assessed at baseline as well as during 12 and 42 months of therapy with 8 mg/m²/week (~0.037 mg/kg/day) recombinant GH (Pharmacia, Dübendorf, Switzerland), administered by daily subcutaneous injections. The fat mass was measured by dual-energy X-ray absorptiometry [22], pubertal stage, height, and weight by standard techniques [23], serum glucose by the standard hexokinase method, fasting insulin by enzyme-linked immunoabsorbent assay (Tosoh, Tokyo, Japan) and, since 1997, by 'Access ultrasensitive' sandwich immunoassay [Beckmann, Zürich, Switzerland; interassay coefficient of variation (CV) = 3.5%], cortisol by chemiluminescence assay (Nichols Institute, Nijmegen, The Netherlands; CV = 7.1%), androstenedione (AD) by ¹²⁵I-RIA (DSL, Webster, Tex., USA; CV = 4.8%), dehydroepiandrosterone (DHEA) also by ¹²⁵I-RIA (DSL; CV = 3.5%) and its sulfate (DHEAS) by chemiluminescence assay (Nichols Institute; CV = 9.0%), and leptin (CV = 8.5%) [24] and IGF-I (CV = 10.1%) [25, 26] as published by the authors. Data for subjects with PWS were judged individually as to whether they were within the age- and sex-related reference range used by the laboratory measuring the respective parameter. Examples of reference ranges for boys and girls together, age 6–9 years are given in table 1 and 2 to facilitate the estimation of mean values in patients with PWS. In particular, reference data for androgens and cortisol in US children were provided by Endocrine Sciences (Tarzana, Calif., USA). We recently described [22] how lean and fat mass were related to normative data established in Caucasian US [27] and in Dutch children [28, 29]. The leptin concentration as well as the BMI (body weight in kilograms divided by the square of height in meters) were compared with age-related data of healthy German schoolchildren, while IGF-I [25] and Insulin [30] levels were compared with data obtained from Swiss and Finnish children, respectively, as published previously [17]. The insulin resistance was assessed by homeostasis model analysis (HOMA-R) and compared with data of healthy adults [31]. Missing values were not due to a specific selection of patients or dropouts, but to shortage of serum as a result of the difficulties encountered during venipuncture in children with PWS or, in 3 patients, to uncompleted investigation intervals as a consequence of delayed start of therapy. For the same reason, the AD levels before therapy could not be included into the evaluation.

The patients continued their energy-reduced diets during the study protocol [21, 22]. No additional medication was administered besides substitution for hypothyroidism in 1 1.5-year-old boy (*L*-tyroxine 50 µg) and for hypogonadism in 3 adolescents after more than 14 months of GHT: 1 14.5-year-old girl receiving ethynyl estradiol 10 µg, 1 14.8-year-old boy treated with testosterone enanthate 100 mg i.m., and 1 16.6-year-old girl receiving Trisequens® (2 mg estradiol, 1 mg norethisterone acetate) [21, 22].

The study has been approved by the Ethics Committee of the Children's University Hospital of Zürich, and informed consent was obtained from the parents.

Statistical Methods

All data were processed by GAS 4.1 of the Institute for Medical Informatics (IMI, Zürich, Switzerland). Because most of the variables showed a skewed distribution, they were transformed to logarithms which resulted in an approximately normal distribution. We had to establish age-standardized values, since all variables, except

Table 1. Adrenal serum steroids in children with PWS (dependent variables, mean \pm SEM)

	Reference range (6- to 9-year-old females and males) ^a	Before therapy	n	12 months after GHT	n	42 months after GHT	n
DHEAS, nmol/l	<1,460	1,630 \pm 500	19	2,410 \pm 610 ^{n.s.}	19	3,340 \pm 710 ^{n.s.}	14
DHEA, nmol/l	0.7–9.1	10.2 \pm 2.9	17	19.6 \pm 4.5 ^(#)	19	22.2 \pm 3.5 [#]	15
AD, nmol/l	0.3–1.7	(3.3 \pm 0.83)	6	2.38 \pm 0.5	18	3.3 \pm 0.53	15
Cortisol, nmol/l	240–690	440 \pm 53	16	260 \pm 30 ^{###}	18	270 \pm 20 ^{##}	17

^a See Patients and Methods for further description of reference data (provided by the Institute of Pediatric Endocrinology, Basel, Switzerland).

^b Between 12 and 42 months of GHT, no significant change of any parameter was found.

(#), #, ##, ###, n.s. p = 0.06, <0.05, <0.01, <0.001, or not significant, respectively, indicate whether the residuals of the hormones at 12 or 42 months, corrected for age at baseline, differ significantly from data before therapy (tested by paired t test).

Table 2. Hormonal and anthropometric covariates in children with PWS (mean \pm SEM)

	Reference range (6- to 9-year-old females and males) ^a	Before therapy	n	12 months after GHT	n	42 months after GHT	n
BMI, kg/m ²	16.55 \pm 2.07 [24]	19.1 \pm 1.2	23	18.7 \pm 1.4 ^{###}	23	18.7 \pm 0.93 ^{###}	19
Fat, kg ^b	5.89 \pm 4.3 [27, 29]	13.1 \pm 3.25	16	12.5 \pm 4.0 [#]	16	11.6 \pm 2.0 [#]	13
IGF-I, ng/ml	161 \pm 61 [25, 26]	101 \pm 15	23	287 \pm 30 ^{###}	22	375 \pm 38 ^{###}	17
Leptin, ng/ml	2.51 \pm 1.16 [24]	13.4 \pm 3.4	23	10.1 \pm 4.0 ^{###}	12	–	
Insulin, mU/l	7.10 \pm 3.70 [30]	6.18 \pm 1.84	18	9.26 \pm 1.40 ^{##}	19	5.13 \pm 1.00 ^{#,†}	16
HOMA-R ^c	1.48 \pm 0.46 [31]	1.30 \pm 0.39	15	1.99 \pm 0.33 [#]	13	1.12 \pm 0.17 ^{n.s., d}	14

For definition of the significance levels see table 1.

^a See Patients and Methods for further description of reference data.

^b Data of body composition shown in detail elsewhere [22].

^c Index of insulin resistance = Ins [mU/L] / 22.5 * e^{-lnGluc[mmol/l]}.

^d Data obtained after 36 months of GHT.

[†] Significance of differences of the residuals at 42 months, corrected for age at baseline, vs. data at 12 months (tested by paired t test).

for cortisol, were clearly age dependent. To do so, we calculated a least squares fit with age as explanatory variable and the logarithm of the variable as the response. This regression was made independently at baseline and at 12 and 42 months of GH treatment. Age-standardized values were then defined as the residuals with respect to each fit. This standardization technique is equivalent to considering age as a covariate. Such standardized variables will be denoted by the subscript 'res' in the following sections of the paper.

For each variable, we also compared values at baseline with values after 12 or 42 months of GHT. In order to distinguish whether the changes observed were solely related to the increase of age or whether they were due to the particular effect of GHT, we calculated age-standardized values after 12 or 24 months of GHT with respect to the baseline regression equation. In doing so, we were able to elim-

inate the age effect from all variables. We then applied paired t tests to assess any significant difference between baseline residuals versus residuals after 12 or 42 months, all being age-standardized by the same baseline equation, hence indicating changes due to the treatment itself. Note that, after 12 or 42 months of GHT, this estimate included the extrapolation of age for the adolescents older than the maximal age of 14.6 years at start. But we obtained essentially the same results by repeating the same calculations without the extrapolated values.

To allow for a sufficient number of subjects being available for statistical calculations, we decided to evaluate data of girls and boys together, because initial pubertal stages of pubic hair, breast, and genitalia were below 3 and because graphical representations did not reveal an obvious gender difference.

In order to investigate the relative contribution of anthropometric (BMI_{res} and $fat\ mass_{res}$) and hormonal variables ($leptin_{res}$, $IGF-I_{res}$, fasting insulin $_{res}$, or $HOMA-R_{res}$) to the levels of $DHEAS_{res}$, $DHEA_{res}$, AD_{res} (this only after 12 and 42 months), and cortisol $_{res}$, we considered regression models with one or two explanatory variables. The relationship between an 'explanatory' – anthropometric or hormonal – variable and the response ($DHEAS_{res}$, $DHEA_{res}$ or cortisol $_{res}$) was characterized by the Pearson correlation coefficient r_{Pea} or, equivalently, by its square, R^2 , expressed as a percentage which represents the proportion of the response due to the explanatory variable. The relationship between two explanatory variables was investigated in the same way. In the regression models with two explanatory variables, we used F tests to assess the joint influence and partial F tests to assess the contribution of each explanatory variable introduced into the model.

Throughout the analyses, $p < 0.05$ was considered significant, whereas we considered to have a trend for p values between 0.05 and 0.10.

Results

Premature adrenarche was observed in 1 girl out of 19 patients (5.2%) before and in 1 girl and 1 boy (10.5%) during GHT. Excluding young children below the age of 3 years, in whom the presentation of idiopathic premature adrenarche is highly improbable, the incidence would rise to 1 of 11 (9.1%) children before and to 2 of 11 (18.2%) children during therapy. High androgen levels, e.g., values exceeding the normal range for gender and age, were found in 47% of the $DHEAS$ and in 35% of the $DHEA$ samples at baseline. This proportion increased after 1 year of GHT to 53 and 63%, respectively; after 3.5 years of GHT, again 53% of the children presented with elevated $DHEAS$ levels and 72% with elevated $DHEA$ levels (data not shown).

Along this line, the mean levels of $DHEAS$, $DHEA$, and AD were higher than our reference ranges at the baseline examination and even more distinctively so after 12 and 42 months of GHT (table 1). However, when the changes of variables were corrected for the effect of age, only the increase of $DHEA$ was significant after 42 months. In fact, the observed increase of $DHEAS$ was due to age. For AD we lacked a sufficient number of measurements before therapy to draw any conclusion. Thus, on average, the increase of adrenal androgens in the group was not clearly associated with the institution of GH treatment. In contrast, the cortisol levels were well within the normal range at the first examination and significantly dropped to the lower normal range during GHT.

The covariates showed several abnormalities, as demonstrated in previous studies [17, 22, 32]. Mainly the fat mass was increased in PWS, and the leptin levels and the

BMI were high (table 2, as a consequence of their strong correlation with fat mass ($r = 0.85$ and $r = 0.75$, respectively, with both $p < 0.01$ and $n = 16$). In spite of adiposity, $IGF-I$, fasting insulin, and insulin resistance ($HOMA-R$) were in the lower normal range, being explained by hypothalamic GH deficiency, before therapy. During the GHT, the increase in $IGF-I$ levels was highly significant, and a significant loss of fat mass was induced, accompanied by a significant decrease of leptin and BMI , all variables having been corrected for age as described in Patients and Methods. The insulin levels significantly rose after 1 year of GHT to fall slightly below baseline levels after 42 months.

Before therapy, significant positive correlations were found between the adrenal androgens $DHEAS$ and $DHEA$ as well as several hormonal parameters, but not with anthropometric variables (table 3).

In the regression models, R^2 was deduced from correlation coefficients (table 3) as indicated in Patients and Methods, and first $DHEAS$ as dependant variable was investigated. 34% of the $DHEAS$ variance was explained by insulin alone, and the bivariate model, when insulin was already in, remained significant, but substantially unchanged by the introduction of BMI or $IGF-I$ (38 or 39% of $DHEAS$ variance explained, respectively; $p < 0.05$, $n = 16$). Therefore, insulin, but not $IGF-I$, had the main influence on $DHEAS$ in this model. The surrogate of insulin resistance, $HOMA-R$, was even more strongly linked with $DHEAS$: $HOMA-R$ alone explained 42% of the $DHEAS$ variability in the univariate model deduced from r_{Pea} (table 3), and this relationship remained similar in the bivariate models additionally including $IGF-I$ ($R^2 = 44\%$, $p = 0.055$, $n = 13$), fat mass ($R^2 = 50\%$, $p = 0.043$, $n = 12$), or BMI (data not shown), all by themselves not being significantly associated with $DHEAS$ (table 3). The covariate insulin was not significantly related to fat mass or body mass, and there was only a trend of $HOMA-R$ to correlate with fat mass or BMI ($r = 0.5$ for both; $p = 0.068$ and $n = 14$ and $p = 0.056$ and $n = 15$, respectively). Because of the small sample, there was only a trend, although the correlations were substantial. Leptin or $IGF-I$ levels were not associated with $DHEAS$, neither alone (table 3) nor in any combination tested (data not shown).

In the univariate model with $DHEA$, 24% of the variance of the androgen was explained by leptin alone, but in the bivariate model neither the introduction of BMI (not significant) nor of fat mass (36%, $p = 0.087$, $n = 14$) significantly increased R^2 , both covariates alone not being significantly associated with $DHEA$ (table 3). Furthermore,

Table 3. Correlations in children and adolescents with PWS before therapy

0 months	DHEAS _{res}			DHEA _{res}			Cortisol _{res}		
	r	p	n	r	p	n	r	p	n
BMI _{res}	0.00	n.s.	19	0.33	n.s.	17	-0.39	n.s.	16
Fat mass _{res}	0.00	n.s.	14	0.22	n.s.	14	-0.40	n.s.	14
IGF-I _{res}	0.22	n.s.	19	0.58	0.016	17	-0.30	n.s.	16
Leptin _{res}	0.20	n.s.	19	0.49	0.046	17	-0.33	n.s.	16
Insulin _{res}	0.58	0.017	16	0.41	0.065	17	-0.28	n.s.	16
HOMA-R _{res}	0.65	0.018	13	0.57	0.045	13	-0.32	n.s.	13

r = Pearson's correlation coefficient; p = associated significance; n = sample size available for the analysis of correlation.

n.s. = Not significant.

Table 4. Correlations after 12 months of GHT

12 months	DHEAS _{res}			DHEA _{res}			AD _{res}			Cortisol _{res}		
	r	p	n	r	p	n	r	p	n	r	p	n
BMI _{res}	-0.10	n.s.	19	0.14	n.s.	19	0.00	n.s.	18	-0.22	n.s.	18
Fat mass _{res}	-0.10	n.s.	14	0.14	n.s.	14	0.27	n.s.	14	-0.48	0.082	14
IGF-I _{res}	0.30	n.s.	18	0.10	n.s.	18	0.51	0.036	17	-0.10	n.s.	17
Leptin _{res}	0.20	n.s.	10	0.82	0.004	10	0.52	0.15	9	0.28	n.s.	9
Insulin _{res}	0.10	n.s.	16	0.00	n.s.	16	0.00	n.s.	15	-0.40	n.s.	15
HOMA-R _{res}	0.10	n.s.	10	0.14	n.s.	11	0.00	n.s.	10	-0.23	n.s.	10

r = Pearson's correlation coefficient; p = associated significance; n = sample size available for the analysis of correlation.

n.s. = Not significant.

the introduction of IGF-I into the bivariate model with leptin slightly improved the relationship, explaining 40% of the variance of DHEA ($p = 0.03$, $n = 17$). In the next model, IGF-I, on its own, significantly predicted 33% of the DHEA variability (table 3). IGF-I with BMI together accounted for 35% of the DHEA variance ($p = 0.051$, $n = 17$). The most significant and additive bivariate model was found when HOMA-R was included in addition to IGF-I, now explaining together 60% of the DHEA variance ($p = 0.01$, $n = 13$). HOMA-R alone accounted for 32% of the DHEA variance, and this relationship was not significantly improved neither in conjunction with BMI nor with fat mass. Insulin was not strongly correlated with DHEA (table 3, $R^2 = 22\%$), but the joint influence of insulin and IGF-I was significant and additive, accounting for 57% of the variance of DHEA ($p = 0.004$, $n = 17$). No significant correlations were found between cortisol and

body composition or hormonal variables before or during therapy (table 3, 4).

After 12 months of GH treatment (table 4), the levels of DHEAS were no longer associated with any independent variable. The levels of DHEA were now only explained by leptin (table 4, $R^2 = 67\%$), and the regression model remained roughly unchanged after the addition of BMI ($R^2 = 74\%$, $p = 0.008$, $n = 10$) or insulin ($R^2 = 78\%$, $p = 0.011$, $n = 9$), but in the bivariate model no other anthropometric or hormonal parameter was significantly associated with DHEA. Since at this time the number of AD measurements was sufficient, the regression model could also be tested for this dependant variable: IGF-I was the only parameter to explain the variance of AD (table 4, $R^2 = 26\%$), and the bivariate model was no longer significant when other variables were brought in. In this connection, it has to be pointed out that in children with

PWS, the covariate IGF-I did not correlate either with lean mass or with fat mass or BMI before or during therapy. Furthermore, the lean mass did not correlate with adrenal androgens at any time. The development of the cortisol levels could not be explained by any independent variable, but there was only a trend for fat mass to be negatively associated with the cortisol levels (table 4).

After 42 months of therapy, no significant explanation was delivered by the independent variables to interpret the development of the levels of androgens (data not shown).

Discussion

In summary, the mean adrenal androgen levels in patients with PWS are elevated above the normal range, as described in healthy obese children [3, 4]. The increased levels of DHEAS, DHEA, and AD may give rise to a precocious growth of pubic hair which then is termed premature adrenarche [33]. The present study presents arguments to support the hypothesis that the dissociation of the adrenal androgen secretion from gonadal maturation in PWS is amplified by obesity and related metabolic alterations, independently of the hypothalamic regulation.

The increase of adrenal androgen levels in PWS, before onset of therapy, is correlated with hormones dependent on body composition and growth, namely insulin, leptin, and IGF-I. The higher the insulin levels were, and, in particular, the more insulin-resistant the subject was, the more the baseline levels of DHEAS and DHEA were increased. These correlations agree with findings in obese children with premature adrenarche [34] or in obese hirsute women [35]. However, insulin and HOMA-R were in the lower normal range, in contrast to simple obesity [1, 36]. Since the insulin sensitivity is increased in the presence of GH insufficiency, it can be deduced that the relationship to androgens occurs at a lower level of insulin in patients with PWS. IGF-I is the second important covariate related to DHEA at baseline and AD during therapy, as in healthy obese children [8, 37] and adolescents [38]. Most importantly, in the regression models, insulin or HOMA-R, in addition to IGF-I, make contributions to explain up to 60% of the variance of baseline DHEA levels. This means that they may represent distinct mechanisms to enhance levels of this androgen. This is corroborated by *in vitro* experiments showing that both insulin [39] and IGFs [40] enhance the enzyme activity of steroidogenic enzymes in human adrenocortical cells.

The data in patients with PWS, showing a correlation between DHEA and leptin levels, confirm not only findings in healthy children [8], but also experimental results on the stimulation of androgen formation via 17/20-lyase at physiological leptin levels in human adrenocortical carcinoma cells [10]. An association between increased circulating androgen and leptin levels has so far been observed only in girls with precocious pubarche [41] and, dependent on BMI, in adult women with polycystic ovary syndrome [42]. We suggest that in patients with PWS, as in healthy subjects, insulin, IGF-I, and leptin mediate the effects of increasing adiposity to enhance the adrenal androgen secretion. In fact, longitudinal studies performed in healthy children or primates have revealed that changes in BMI or related hormones precede adrenarche [43] or puberty [44] before steroid hormones are secreted. Normally, the leptin and IGF-I levels both increase before puberty and might provide the brain with information on body composition and size to start pituitary, adrenal, and gonadal maturation [43, 44].

During GHT, the DHEA concentration significantly rises, potentially due to the increase of insulin and IGF-I levels [40]. Therefore, GHT is likely to enhance the risk of premature adrenarche. However, most associations observed previously disappeared, except for those between leptin and DHEA or IGF-I and AD. The relationships observed at baseline may in part be offset during therapy by the strong, direct effects of exogenous GH on IGF-I concentrations, insulin resistance, and insulin levels [32].

Although the elevation of androgen levels in children with PWS is correlated with hormones linked with the body composition, such as insulin, leptin and IGF-I, no associations are found between androgen levels and adiposity or the anthropometric variables BMI, fat, or lean mass, either before or during therapy. This observation contrasts results in healthy children with and without simple obesity, even if the studies were carried out by similar methods [8]. In healthy children, the pubic hair growth is advanced with increased BMI [45], and the serum DHEAS levels are correlated with the BMI [37, 38]. Furthermore, the steroid excretion is increased in obese children [46], and the androgen excretion correlates with the lean mass [47]. The dissociation between anthropometric variables and increased androgen levels in patients with PWS suggests that the regulation of body mass and body composition is altered in PWS, as described by other authors. There is a peculiar, central subcutaneous fat deposition in patients with PWS [48], but the visceral fat compartment is relatively reduced [20] as compared with simple obesity. The reduction of the metabolically active

visceral fat is specific to PWS and is compatible with the observations that insulin levels in these patients are low, even after 3 years of GHT [32], and that the triglyceride levels are normal [49].

The levels of circulating cortisol are normal, in contrast to adrenal androgen levels, and do not correlate with any other covariate, as described in healthy obese children [50]. Since the cortisol levels were normal and, during GH treatment, decreased within the reference range, independently of the increase of adrenal androgens, we deduced a grossly normal hypothalamic-pituitary regulation of the adrenal gland in PWS. However, in theory, the cortisol metabolism could be enhanced by changes induced during GHT, e.g., insulin increase, but the present outpatient setting did not allow for a collection of 24-hour urine samples from the handicapped children. The present study was not designed to examine the hypothalamic-pituitary-adrenal axis exhaustively. Therefore, its function in PWS will have to be assessed in further, more sophisticated studies.

In conclusion, the typically delayed gonadal development in PWS may be contrasted by elevated androgen levels and premature adrenarche. We suggest that these represent epiphenomena of hormonal changes induced by

adiposity rather than being the consequence of the assumed hypothalamic defect in PWS. However, in PWS, specific alterations change the pattern of the pubertal development: (1) the effects of insulin or IGF-I on circulating androgens are exhibited at lower levels than in subjects with intact hypothalamic function; (2) the associations between anthropometric and hormonal variables are disrupted, possibly by the basic defect of the regulation of the body composition, and (3) the gonadal development may be impeded due to the hypothalamic defect.

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